MICROSCOPY.[1]

SOME ANATOMICAL AND HISTOLOGICAL METHODS.[2]—*I. A modification of Semper's method of making dry preparations.*—While it may be true that in many cases the preparations made according to Semper's method have an appearance similar to a gypsum model, they quite often present a dingy, weatherbeaten aspect that is by no means agreeable. The thin membranes and the connective tissues of dissections are left in a loose, wooly condition that grows worse by handling.

The microscopist completes his work by mounting his preparations in a solution of balsam. In like manner Semper's method may be completed by saturating the preparation with some solid that would fill up the pores, bind the parts together and restore the natural appearance. The solid which I have employed for this purpose is a mixture of Canada balsam, paraffine, and vaseline, but it is probable that a soft paraffine will in most cases do quite well. It is necessary that the mixture shall melt at about 46° C. (115° F.). It will be seen that the preparation is treated just as the microscopist treats an object when he wishes to obtain a consecutive series of sections. While yet saturated with the turpentine, it is to be immersed in the mixture, heated a little above the melting point and kept there until all the turpentine has been replaced. In many, if not in most cases, however, the turpentine may be allowed to evaporate before the preparation is put into the melted paraffine mass. The latter then quickly penetrates the tissues and the work is simplified. The preparation is then to be kept in an oven vessel warm enough for the excess of paraffine to melt and drain off. It may then be wrapped in cloths or in bibulous paper until the whole of the paraffine mixture adhering to the outside has been dried off.

The advantages to be derived from pushing the process to this stage are the attainment of a greater degree of firmness and strength in the specimen, the obviation of the bleached appearance assumed on the escape of the turpentine, and the restoration of the natural colors. Probably any colors will reappear that will endure immersion in alcohol. In the case of anatomical preparations made in the way described, injected vessels show to advantage. I have also prepared specimens of lizards, small turtles, fishes, mussels and earthworms; and whenever the tissues have been thoroughly saturated with the wax mass, the results have been satisfactory.

II. A method of making double injections for dissecting purposes.—A brief notice of Professor H. F. Osborn's method for double injections appeared in *Science Record*, II, Feb. 15, 1884, p. 84. His plan appears to have been to fill the whole vascular

[1] Edited by Dr. C. O. WHITMAN, Mus. Comparative Zoology, Cambridge, Mass.
[2] By Professor O. P. Hay, Indianapolis.

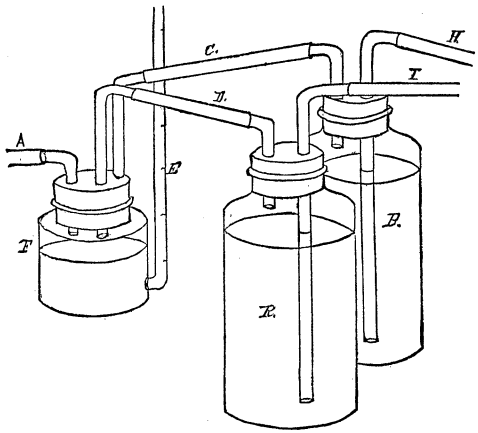
system with a thin colored injection mass, as in making an ordinary injection. When this has passed through the capillaries and well filled the veins, there is forced into the artery a differently colored *plaster mass* which pushes the previously injected thin mass before it until the plaster has reached the capillaries, where its onward movement is arrested. For a year or more before Osborn's notice was published, double injections based on the same principle had been made by the writer. As practiced by myself, a canula was fitted into the aorta of a cat, and a gelatine mass colored with carmine was injected until it was seen to flow from the right side of the heart; then the tube conveying the red mass being detached, a tube conveying a blue gelatine mass was slipped over the same canula, and the pressure again applied. Into this blue mass had been mixed thoroughly a quantity of starch, preferably from wheat. This starch-bearing mass pushed the carmine mass before it until the starch grains entered the capillaries and effectually plugged them up. The arteries were thus left blue and the veins red, and so well was the work accomplished that a lens of considerable power had to be used to discover any admixture of the colors in the smallest vessels of thin membranes. The first mass injected need not be unusually thin.

The capacity of the capillaries is so great, as compared with that of the arteries, that any commingling of the two colors is concealed in them. Carmine is used for the veins because of the ease with which it may be prepared, its permanence and the facility with which it passes through the capillaries. On the other hand, the gelatine for the arteries may be colored with the coarser pigments, such as Prussian blue or ultramarine. The latter furnishes a beautiful blue. Vermilion is not suitable for the first injected mass, since on account of its high specific gravity it readily sinks to the lowest side of the vessels, drags behind, and causes a commingling of the colors. An additional reason for filling the veins with red rather than with blue is found in the agreeable and natural color given to the preparation.

Of course a mass of plaster of Paris injected after a gelatine mass will drive it until the plaster reaches the smallest vessels, thus producing a double injection. The starch mass recently proposed as a filling for blood-vessels will readily lend itself to the production of a double injection according to the method detailed above.

III. A method of producing double injections for histological purposes.—So far as I am aware the usual method of producing a double injection of the blood-vessels preparatory to making sections for the microscope, is to inject first a gelatine mass of one color into the artery until the increasing pressure gives notice that the mass is entering the capillaries, and immediately after to inject a differently colored mass into the vein. The injection be-

ing thus accomplished one of two things, it seems to me, is likely to happen; either the vessels will not be well filled or the mass intended for one set of vessels will be driven through into the other. To avoid these accidents I have practiced the method of filling both sets of vessels at the same moment and under exactly the same pressure. This pressure is kept low at the beginning so that all the arteries and veins shall be thoroughly filled before either mass begins to enter the capillaries. Then as the pressure is increased the differently colored masses meet each other in the capillaries; and if the pressure on each is equal, the vessels may be filled as full as compatible with safety without danger of either color being driven from one set of vessels into the other. The way in which this result is accomplished will be understood better by reference to the accompanying drawing. The desired press-



Double-Injecting Apparatus.

ure is secured by allowing a stream of water from a hydrant or from an elevated cistern to flow into a tight vessel. A two gallon petroleum can does quite well. As the water flows in the air is forced out through a rubber tube, *A*, into the wide-mouthed bottle, *F*, whose tightly fitting cork gives passage to two other glass tubes. These extend below just through the cork and above connect respectively with the rubber tubes *C* and *D*. Into the side of *F*, near the bottom is fitted another tube, *E*, reaching to a height of ten inches or more, open above, and graduated into inches. If preferred, this tube may also pass through the cork and extend down well into the mercury with which *F* is partly filled. *B* is a bottle of suitable size in which is contained a blue injection mass for filling the veins, and *R* a similar bottle containing a red mass for the arteries. The interiors of these bottles are connected with the bottle *F* by the tubes *D* and *C*. Each of the bottles, *B* and *R*, has a tube which, starting from near the bottom, passes through the cork, and is, a little above this, bent at right angles. With these are connected the rubber tubes, *H* and *I*.

Now when water is allowed to flow into the reservoir mentioned above, the air is forced out through *A* into *F*, and thence along the tubes *D* and *C* into *B* and *R*. As soon as the pressure in these bottles becomes sufficiently great, the liquids which they contain will be driven out through the tubes *H* and *L*. If there should be any obstacle to the escape of these fluid masses, the pressure in all the vessels will rise and be registered by the height of the mercury in *E*.

If now it is desired to inject, for instance the kidney of a pig, a canula made of a glass tube must be fitted securely into the renal artery and a similar one into the renal vein. The canulæ must be of such a size that the rubber tubes, *H* and *I*, will fit them well. Heat the gelatine masses in the bottles, *B* and *R*, to the proper temperature and keep them so heated until the injection has been finished. Special care must be taken with the tubes, *H* and *I*, to prevent the gelatine passing through them from becoming frozen. Now having clamped the tube, *H*, have an assistant turn on a small stream of water until the gelatine begins to flow slowly from *I*. If the diameter of the canula is not too small it may be held with the free end directed upward and filled with gelatine allowed to drop from the mouth of *I*. Then slip *I* over the canula. Unclamp the tube, *H*, and when the gelatine from *B* has begun to flow, slip it over the canula inserted in the vein. Then increase the pressure gradually until it has reached as high a point as experience has taught to be safe for the organ operated on.

By means of this apparatus, which will require the expenditure of only a few cents and a little ingenuity, double injections may easily be made of any organs whose veins are not provided with valves. I have made injections of the kidney whose arteries and glomeruli became uniformly filled with the red mass and whose veins and the system of capillaries surrounding the renal tubules became filled with the blue. The lungs and the liver are easily and successfully injected. I have been less successful in injecting the organs that send away their blood current through the portal vein; but I have no doubt that they too may be injected.

Triple injections of the liver may be made by first injecting the hepatic artery with a green mass until the whole liver assumes a green tint, and afterwards injecting the portal vein and the hepatic vein with red and blue as above directed.

The same apparatus may be employed to make either single injections or the double injection described under the second head of this paper, by simply clamping one of the tubes, *C* or *D*. As a matter of course care must be taken that all the corks fit tightly in the bottles, otherwise the internal pressure may force them out at the very moment when an accident will do the most damage.